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Final Reports #4

PROTEIN FROM WHEY

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INDUSTRIAL WASTE DIVERSION PROGRAM
FINAL REPORTS # 4

Protein From Whey

Report prepared for :

Waste Management Branch
Ontario Ministry of the Environment

JUNE 1991

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PROTEIN FROM WHEY

Report prepared for:

Waste Management Branch
Ontario Ministry of the Environment

In consultation with

Industrial Program Unit

Report prepared by:

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DISCLAIMER

This report is in partial fulfillment of conditions of a grant given to Protose Separations Inc. by the Ministry of the Environment under the Industrial Waste Diversion Program. The report was prepared by Protose Separations Inc. and documents results of work for which the Ministry of the Environment provided financial assistance.

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PROTOSE SEPARATIONS INC.

Production of Protein from Whey

Introduction

Protose Separations Inc. (Protose) is an Ontario company formed to produce on a commercial scale, pure whey protein isolate and pharmaceutical grade lactose from cheese processing wastes.

Cheese whey represents a serious disposal problem in Ontario, where each month there are a total of 46 cheese plants either dumping or feeding to animals, 19,553,000 kg of whey.

This report outlines the experimental work conducted by Protose and funded by The Ministry of The Environment and Protose. The experimental and subsequent analytical work was conducted at the following locations by Protose personnel working with the resident experts as noted:

- The Ontario Centre for Farm Machinery and Food Processing Technology, Chatham, Dr. Peter Chang;
- Agriculture Canada Food Research Centre, Ottawa, Dr. H. W. Modler;
- University of Manitoba Food Science Department, Winnipeg, Dr. E. D. Murray;
- Griffith Laboratories Limited, Scarborough, J. Holmes, V.P. Technical;
- IWT-Himsley International Limited, Toronto, J. Bennet, B. Glover.

The scope of the work performed was significantly greater than originally planned due to uncertainties that surfaced during the experiments. However, sufficient confirmation of the ion exchange and process technology was obtained to warrant proceeding on to the pilot plant scale.

Summary and Conclusions

The testing program conducted by Protose at the Ontario Technology Centre, and the Agriculture Canada Food Research Centre in late 1987 had the following objectives:

1. To examine representative sources of Eastern Ontario whey for suitability of use in commercial protein extraction and lactose crystallization.

2. To establish that the continuous moving bed (CMB) process utilizing ion exchange is superior to the batch process in respect to operating efficiency, recovery rate, and economics.

3. To determine the physical performance of the ion exchange cellulose under the demands of the CMB process.

4. To establish the level of reduction or elimination of the pollutants in the waste whey steam using this process.

5. To determine the efficiency gains in the protein extraction process achieved through the use of demineralized whey.

6. To produce a protein product with the purity and functional properties required for industry acceptance and commercial success.

The outcome of this test program firmly established that high quality protein could be produced from Eastern Ontario waste whey streams with the virtual elimination of associated pollution. It was also determined that the CMB process was far superior to the batch process in the adsorption cycle but inferior in the desorption cycle. Following a post test program analysis IWT - Himsley International Limited (IWT) feels the desorption problem has been identified and can be overcome. Due to subsequently identified factors the protein product lacked the purity (87% dry basis vs 97% target) expected however functional property expectations were fulfilled.

The evidence to support proceeding with demineralization was inconclusive. More work will be required in this area.

In conclusion the test program has established a reasonably high degree of certainty that the CMB process utilizing ion exchange cellulose works. However prior to proceeding with a full scale commercial plant, a pilot plant would have to be constructed to eliminate uncertainties in the desorption process, provide an operating environment for further demineralization work , allow large scale lactose crystallization, fully charactarize effluent compositions and finally to convince potential customers that a pure protein (97%) can be produced on a continuous basis.

The Continuous Moving Bed (CMB) Process

The batch reactor process was employed both in the original experimental work and the sole commercial plant in existence for the production of high purity protein and USP grade lactose. Protose and IWT, upon the study of this and other alternatives concluded that the CMB process would be a far more efficient and ultimately commercially viable process. IWT is a world leader in the development of the CMB process in other industries and it is felt by both IWT and Protose that the ion exchange cellulose lends itself to this process.

A summary of the advantages of the CMB process are:
-decreased equipment size, capital costs
-decreased energy consumption
-decreased post extraction processing
-decreased chemical consumption
-decreased waste volume
-increased throughput
-increased overall protein recovery
-increased level of automation
-increased market security (patent protection)

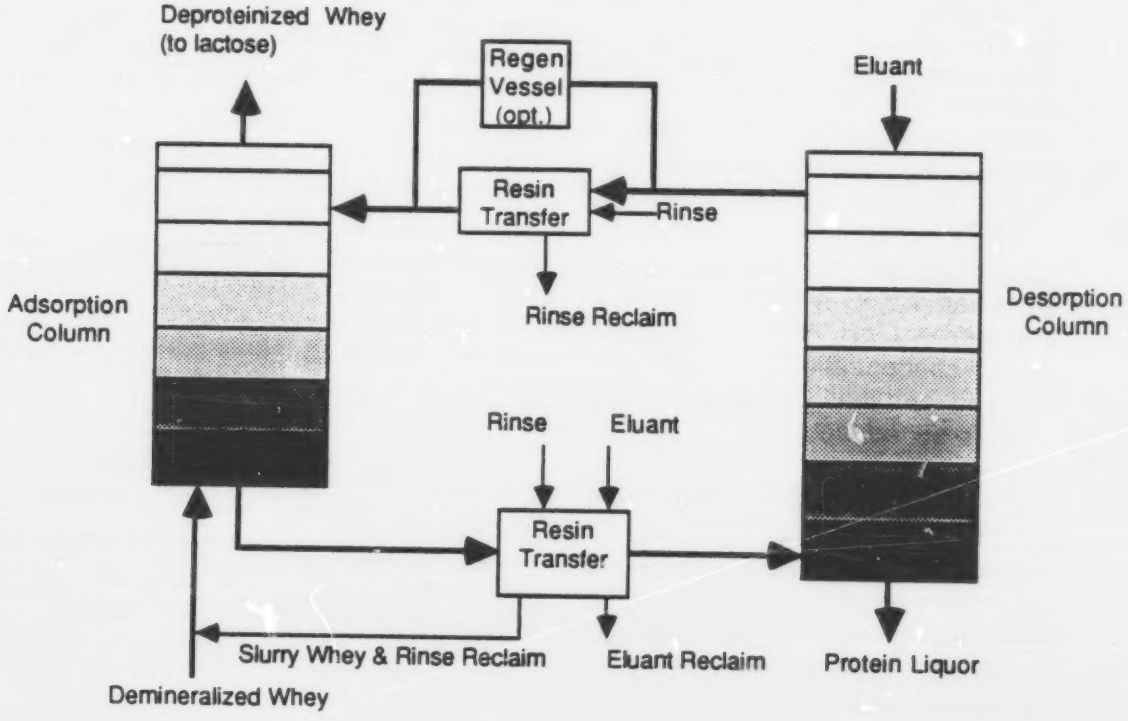
In pursuing the CMB process Protose is attempting to not only significantly reduce the unit costs of high purity protein manufacturing but also develop a patentable process for market

protection. This will be essential in maintaining competitiveness in this large, aggressive and constantly improving world market.

Technology Description

The CMB system consists of an adsorption column, a desorption column and two media transfer chambers. (see Figure 1) Media in the adsorption column is fluidized by the upward, countercurrent flow of whey. When the media at the bottom of the chamber has taken up its full capacity of protein it is slurried out into the transfer chamber and a fresh, regenerated batch added at the top of the column. This design ensures maximum adsorption efficiency as partially loaded media at the bottom of the column is exposed to the highest level of protein in solution while media with full capacity available sees the lowest level. Thus the adsorption side of the equilibrium equation is favoured throughout the column. In effect, the fresh media atop the column acts as a scrubber for residual protein in solution.

Figure 1: Continuous Moving Bed (CMB) Ion Exchange Process



The column height is designed so that media transfer occurs just prior to protein breakthrough at the outlet. Saturated media is slurried in whey feedstock to the transfer vessel. This whey and the subsequent rinse water is then returned to feed storage for protein recovery. The size of the media transfer is determined by the kinetics of the adsorption reaction.

For desorption the media is mixed with eluant and slurried into the bottom of the column, lifting the existing media as a plug. As the media settles , the slurry liquid is displaced back

into storage for reuse. Counter current eluant flow then begins down the column. As with adsorption the transfer equilibrium is favoured through the column and high levels of protein concentration may be achieved. Stripped media at the top of the column is slurried into a transfer chamber, rinsed and returned to the adsorption chamber. An optional second chamber allows isolation of individual batches of media for prolonged, rigorous cleaning and regeneration without disrupting the process flow.

There are a number of distinct advantages which CMB holds over batch extraction. In the batch process 80% of the cycle time is spent in rinsing and desorption stages. With CMB all of this is carried out off-line from the adsorption step. This combines with a fourfold increase in protein load/KG of media to result in a twenty fold decrease in adsorption vessel size.(2500 l vs 50,000 l for a 500,000 l/day plant) The continuous process permits on-line pasteurization and demineralization, eliminating the requirement for large, intermediate surge tanks. Plant shutdown for caustic regeneration of the media every 20 batch cycles is eliminated and the design is ideal for automated CIP.

Improvements in product quality are also anticipated. In a batch process rinsing is accomplished primarily by a dilution process, whereas the CMB flow through approach achieves a higher efficiency with a smaller rinse volume. Reduced volumes translate into lower concentration costs for lactose crystallization while better rinsing yields a higher purity protein product. The output protein concentration is higher (6-20% vs 1.2% for batch) reducing the time, energy and equipment requirements for product concentration prior to drying. Reduced processing results in better functional properties for the end product.

Adsorption: In order to properly design the adsorption bed, the concentration profile of adsorbed protein along the length of the bed is determined. This profile is affected by whey pretreatment, pH, flow rate and temperature conditions. The optimum system has the protein concentration in the effluent from the first "batch" of media equal to at least 95% of the feed and the last batch of media exhibiting no protein breakthrough. It has been predetermined that pH 3.0 is an optimum operating level.

In a theoretical system protein molecules occupy each available ion exchange site as they pass through the column until saturation is achieved and then nonadsorbed material breaks through the column outlet in a well defined, flat front. In reality this does not occur because whey is a mixture of proteins, each exhibiting different adsorption rates. Thus, a gradual increase in breakthrough is observed until saturation is finally attained. The width of this front determines how many stages or "batches" of media are required in the adsorption bed.

Protein in demineralized whey adsorb's at a higher efficiency than in raw whey because of reduced competition for ion exchange sites with minerals in solution. The effectiveness of using demineralized whey that has been adjusted to pH 3.0 with decationized whey (pH 1.35) is being examined to determine if chemical savings are greater than any losses in efficiency.

Various operating temperatures have an effect on adsorption efficiency. Since the process is based on mass transfer, a higher temperature improves results. These improvements must be weighed against equipment sizing, energy costs, opportunity for bacterial growth

and throughput considerations.

Flow rate also has an effect on adsorption efficiency. A slower rate will enhance mass transfer within the media and improve results. This is balanced against sacrifices in throughput time to determine the optimum balance.

Desorption: Protein is desorbed from the media when it acquires a negative charge by exposure to a basic eluant. In a stirred batch reactor, sodium hydroxide is added until all the protein is neutralized and then a small excess to bring the pH to 9.0. This continuous titration is not feasible in a column arrangement, thus the pH will drop through the column as basic eluant is consumed by protein neutralization. All the proteins of concern carry a slight negative charge above pH 7.0, however the rate of desorption increases with higher pH. A necessary consideration is that prolonged exposure to pH greater than 10 may affect certain functional properties of the product or cause gelling. A specialized mechanism for control and delivery of caustic is required for efficient column desorption.

Process Simulation: The CMB process is simulated using a series of one litre columns as media transfer "batches". This involves plumbing columns in series and cycling them to duplicate the resin transfer used in a full scale process. The experiment utilizes the conditions optimized in the single column studies to process approximately 800 litres of whey.

In addition to recovering the protein eluate for product production, the deproteinized whey is collected and frozen for future studies on lactose recrystallization.

Product Processing: The combined eluate from the process simulation is spray dryed directly if sufficient solids concentrations are attained (20%). If necessary the protein liquor can be ultrafiltered to reach the required solids level for drying.

Experimental Results and Discussion

Part 1 - Column Production of Protein

General Protocol: All adsorption and desorption experiments utilized a 2.5" X 12" column packed with 1 litre of swollen ion exchange media. The same resin was used for all experiments with regeneration between uses. The column was controlled at the appropriate temperature and influent whey was passed through a heat exchanger to equilibrate it to column temperature. The pH of the column effluent stream was recorded every minute. Samples (150 ml) were collected every three minutes for analysis. All flow rates were converted to column bed volume (Bv) equivalents (1 Bv= 1 litre) for presentation of data. Equipment limitations prevented the use of conditions exactly as outlined in the original proposal. (e.g. maximum temperature attainable on column was 32°C, minimum 20°C) All protein, carbohydrate (lactose) and fat levels were determined on a Superscan instrument using the principle of infrared absorbance unless otherwise noted. Protein readings represent total nitrogen (true protein nitrogen + non-protein nitrogen) unless otherwise specified. All absorbance measurements were taken at 280 nm with a 1 cm pathlength cell after a 10 fold dilution of the sample.

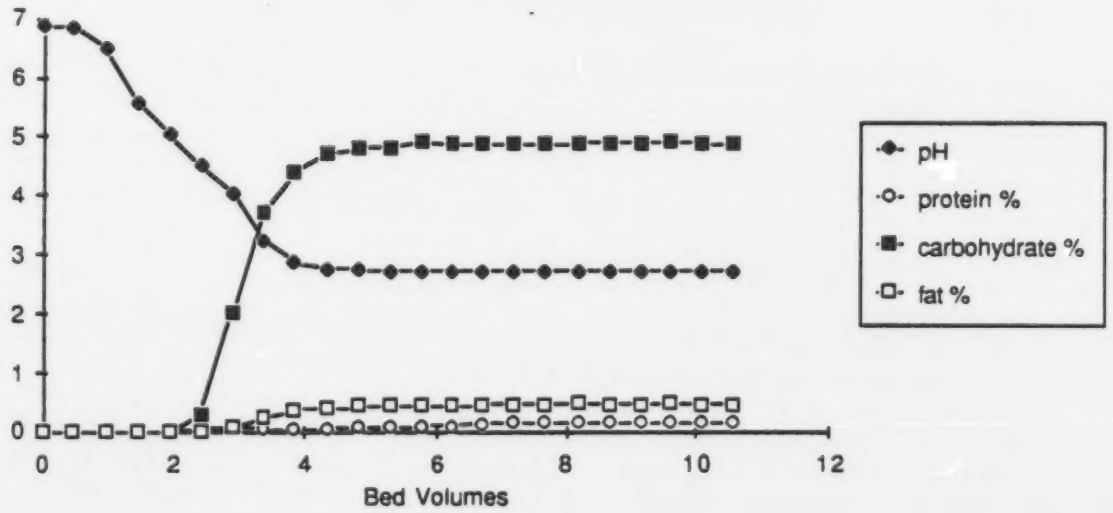
Adsorption:

Experiment 1

Conditions:	Whey Source	Riverside specialty
	whey initial pH	6.13
	whey adjusted pH	3.0
	column temperature	32°C
	flow rate	10 Bv/hr

The analytical results are shown in figure 2. It can be seen that approximately 4 bed volumes were required before the column reached equilbrium. The front of material passing through the column (see carbohydrate for example) was well defined indicating an evenly packed bed. The width of this front was relatively broad (2 Bv) compared to column size which may be accounted for by the use of support screens as opposed to distributor plates which would have minimized mixing at the column inlet. The width of the front decreased to approximately 1 Bv for all subsequent runs which could indicate some non-specific adsorption by the virgin media during this initial whey exposure. This was expected based on previous literature reports. Analysis at equilibrium (10 Bv) indicated a 70 % removal of total nitrogen and no detectable retention of carbohydrate or fat.

Figure 2: Adsorption of Protein - Experiment 1

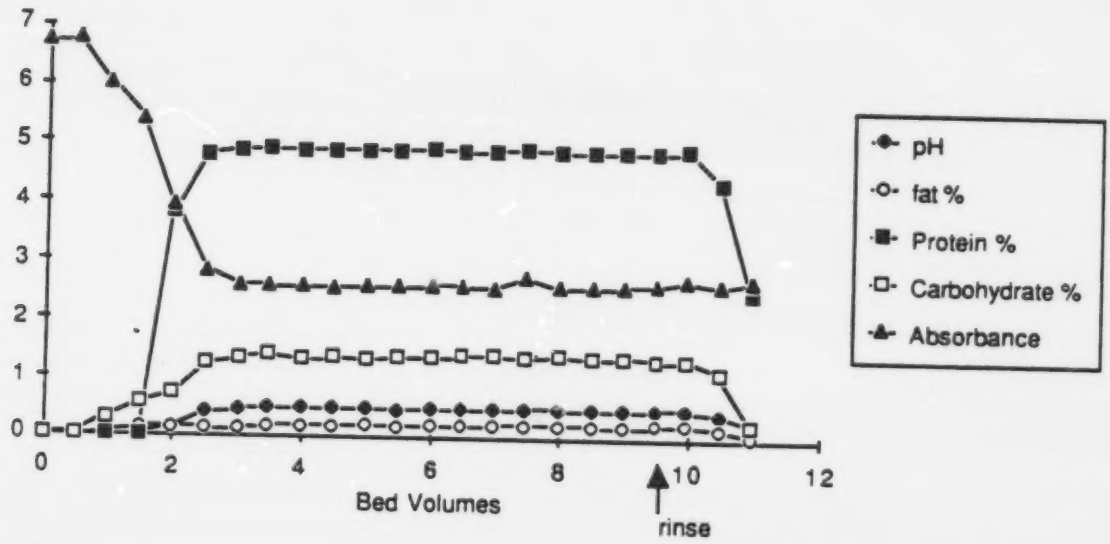


Experiment 2 - effect of lower temperature

Conditions:	Whey Source	Riverside specialty
	whey initial pH	6.13
	whey adjusted pH	3.0
	column temperature	20°C
	flow rate	10 Bv/hr

The analytical results are shown in figure 3. Approximately 2 Bv were required to reach equilbrium, with a front width of 1 Bv. This is significantly reduced from the previous experiment, most likely due to the elimination of non-specific binding sites. Analysis at equilibrium indicated a 64% removal of total nitrogen and no significant retention of carbohydrate or fat. This 6% decrease in effeciency compared to exp. 1 can be explained by the use of the lower temperature (20° vs 32°). The absorbance readings paralleled the protein readings in appearance, however the two methods gave significantly different quantitative results. At equilibrium, residual protein was 0.22% by Superscan whereas the absorbance reading indicated 0.64% based on a standard curve prepared from commercial whey protein concentrate.

Figure 3: Adsorption of Protein - Experiment 2



Experiment 3 - effect of slower flow rate

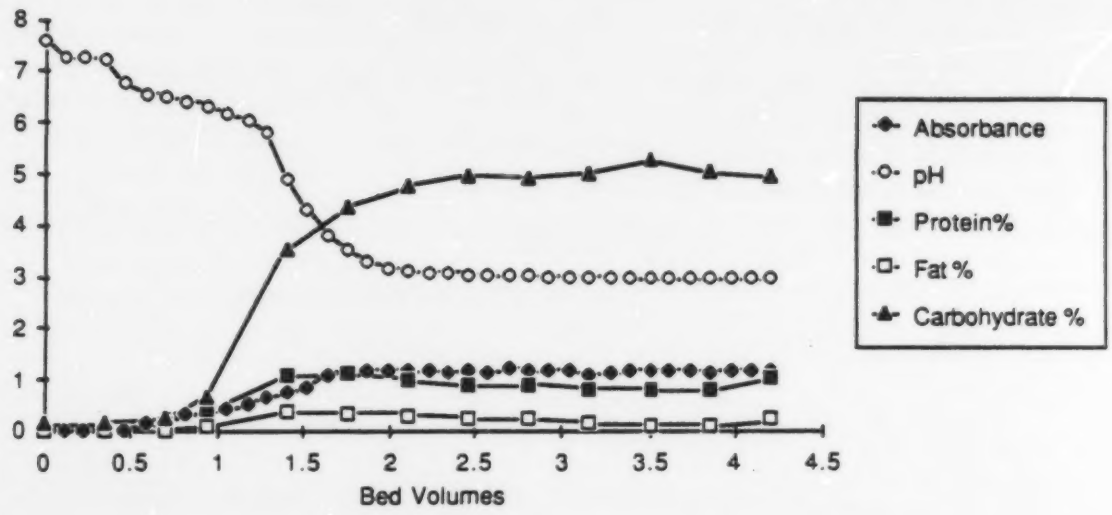
Conditions:

Whey Source	Riverside specialty
whey initial pH	6.13
whey adjusted pH	3.0
column temperature	32°C
flow rate	2.5 Bv/hr

The analytical results are shown in figure 4. All protein samples were adjusted to pH 12 before measurement on the Superscan. (see separate discussion on Superscan analysis) Approximately 2 Bv were required to reach equilbrium, with a front width of 1 Bv. The front was not as well defined as in previous experiments apparently from irregularities in the bed formation. Analysis at equilibrium indicated a 75% removal of total nitrogen and no significant retention of carbohydrate or fat. The 5% increase in efficiency compared to experiment 1 is a result of a slower flow rate allowing a longer contact time with the media. The absorbance readings again paralleled the protein readings in appearance however the two methods gave significantly different quantitative results. At equilibrium protein analysis was 0.90% by Superscan whereas the absorbance reading indicated 0.32%

based on a standard curve prepared from commercial whey protein concentrate.

Figure 4: Adsorption of Protein - Experiment 3



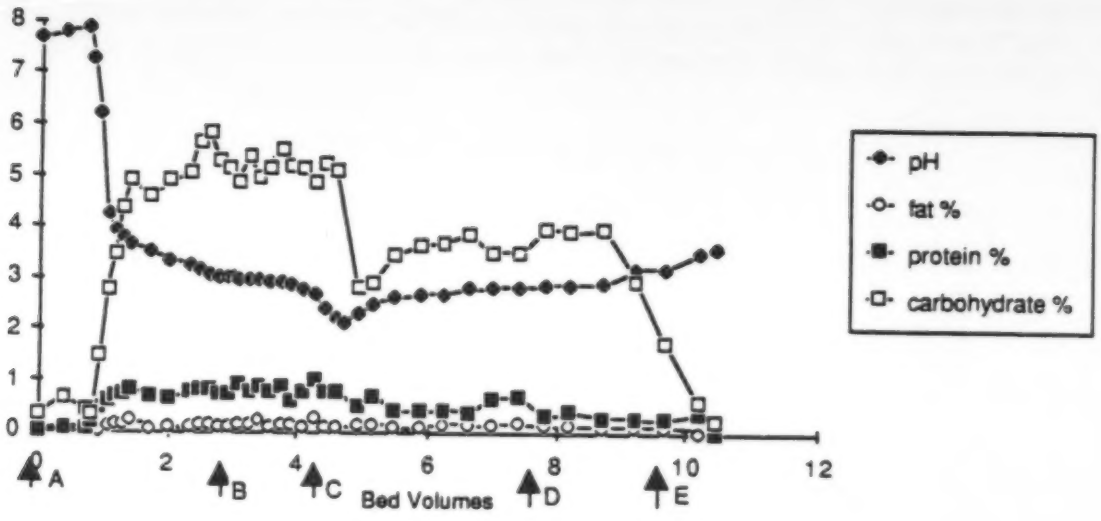
Experiment 4 - effect of demineralization

Conditions:		
	Whey Source	Harold white cheddar
	whey initial pH	5.0
	whey adjusted pH	see text
	column temperature	32°C
	flow rate	2.5 Bv/hr

In order to accentuate any differences in adsorption with demineralized whey the column was first pumped with 3 Bv of pH 3 whey, then 1.5 Bv of pH 2 whey followed by 3.5 Bv of demineralized whey adjusted to pH 3.0 with decationized whey.

The analytical results are shown in figure 5. All protein samples were adjusted to pH 12 before measurement on the Superscan. (see separate discussion on Superscan analysis) There was no detectable difference between % adsorption with pH 2.0 whey vs pH 3.0 whey, however the degree of observed protein removal with demineralized whey was increased. Analysis showed the average removal of "true" protein with regular whey to be 71%, while demineralized whey exhibited a 93% true protein removal. True protein was determined as total nitrogen minus non-protein nitrogen by measurement before and after trichloacetic acid precipitation.

Figure 5: Adsorption of Protein - Experiment 4



A - pH 3.0 whey, B - pH 2.0 whey, C - demin whey, D - rinse, E - eluant

General Discussion:- Adsorption

The hydrodynamic properties of the cellulosic media were shown to be very compatible with the CMB process in terms of fluidization, settling rates, pressure drop and flow rates achievable. The efficiency of the column process for adsorbing protein from solution appears to be higher than that of the batch process.(70% total nitrogen removal vs 50% for batch) As predicted, efficiency is increased by raising temperature or lowering flowrate. Demineralizing the whey appears to yield significant improvements in protein adsorption. This was supported by subsequent analysis of the effluent wheys used for lactose crystallization where the demineralized sample had a 20% lower protein content. Further work is necessary to verify that this protein is removed by the ion exchange media and recovered as opposed to being lost onto the demineralization resins. An unexpected observation was an apparent 25% reduction in lactose concentration by demineralization which requires further investigation.

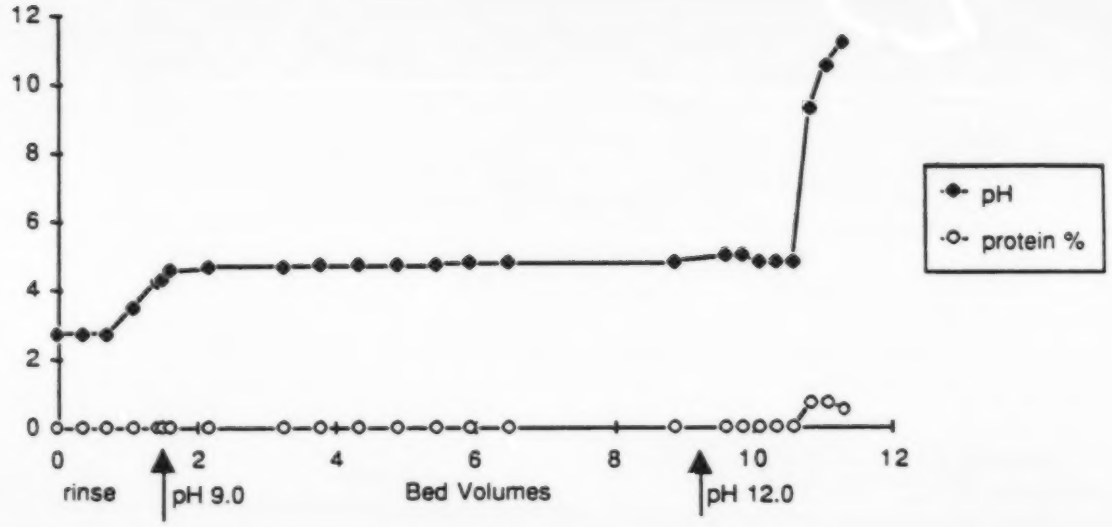
Desorption

Experiment 5

A protein loaded column was rinsed with 2.0 Bv of water at 30°C at a flow rate of 2.5 Bv/hr. This was followed by 8 Bv of water adjusted to pH 9.0 with NaOH. Because this volume failed to overcome the buffer capacity of the protein (indicated by the pH being maintained at the isoelectric point of whey proteins) on the column and had exceeded any commercial feasibility, the pH of the eluant was raised to 12.0 to pull the protein of the column. Although the desired increase in pH was obtained, it resulted in the formation of large quantities of white gel within the column. As can be seen from the results in figure 6,

very little protein was eluted from the column. Analysis of the gel revealed it to be protein, and it was determined through subsequent bench work that exposure of the high concentrations of protein on the column to pH > 11.0 had resulted in the gelling effect. The column was cleaned by washing with large quantities of water, followed by acid, followed by concentrated caustic followed by more water until the pH had returned to 7.0.

Figure 6: Desorption of Protein - Experiment 5



Experiment 6

In an attempt to minimize the volumes of eluant required, and consequently the dilution of eluted protein, it was decided to initially load sufficent caustic to overcome the buffer capacity of the protein and then elute it with pH 9.0. A column was prepared as in exp. 5 and 100 ml of 1.25 N NaOH pumped onto the column, followed by 5 Bv of pH 9.0 solution. Again gelling occured within the column as the pH rose quickly to 12. As can be seen from the results in figure 7, slightly more protein was eluted by this method than the previous but the amount recovered was still < 15% of the total material on the column. Figure 8 shows the comparison of UV absorbance to the superscan readings. Although they track well, the concentrations calculated from the absorbance measurements were approximately 2.5X lower than those determined by the Superscan. No detectable fat or carbohydrate was eluted with the protein.

Figure 7: Desorption of Protein - Experiment 6

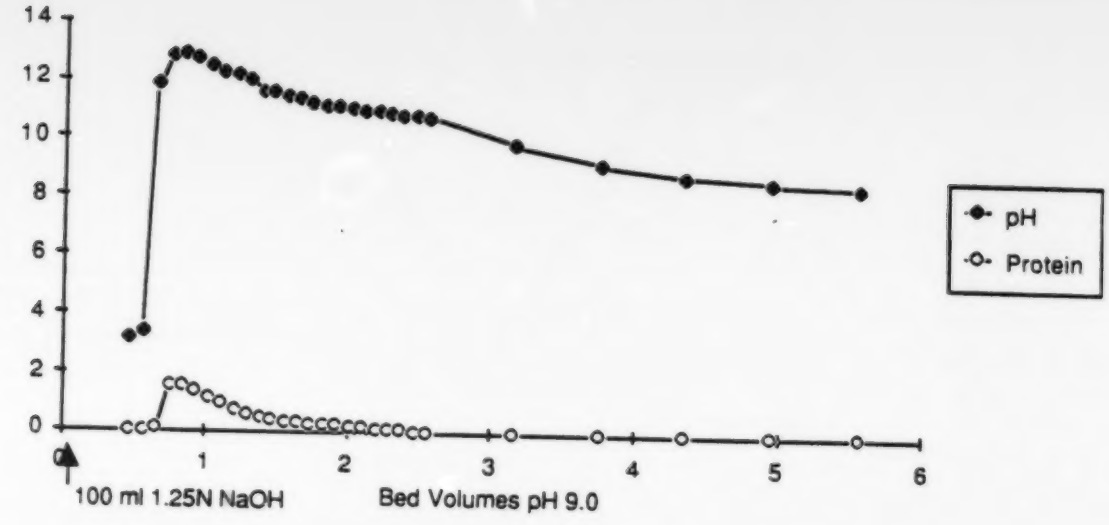
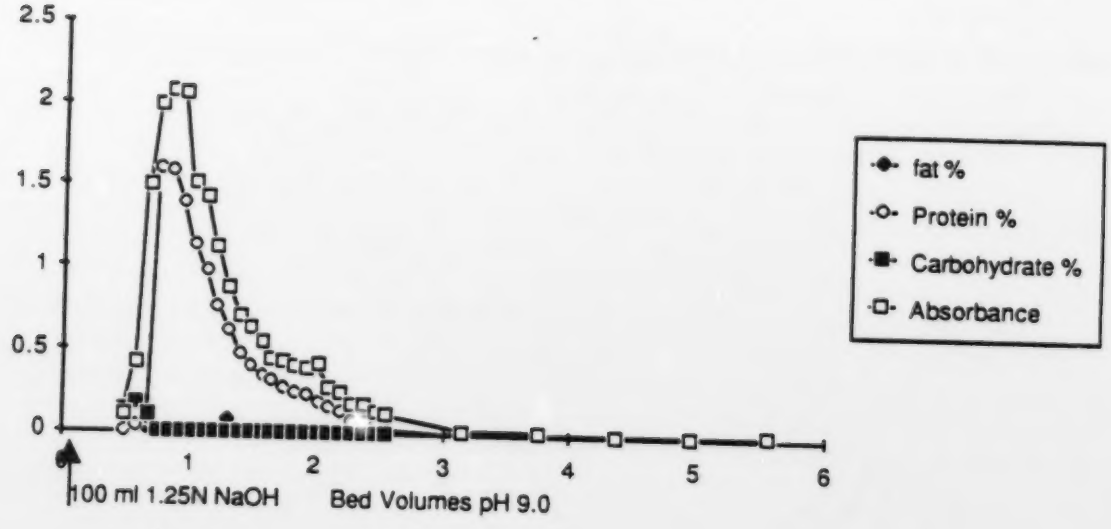


Figure 8: Desorption of Protein - Experiment 6



Experiment 7

In a further attempt to identify the buffer capacity of the protein, another column was prepared as above and pH 10.5 solution pumped at 2.5 Bv/hr. When this failed to overcome the isoelectric point after 14 Bv, pH 12.0 was used to raise the pH. Although the pH did not immediately exceed 11.0 (figure 9) significant gelling had occurred before any protein was eluted from the column. As shown in figure 10, even smaller amounts of protein were eluted (< 3%) than in previous experiments, possibly indicating that the longer the material is on the media the more difficult it may be to remove. The correlation

between protein and absorbance measurements was similar to that in exp. 6. A small non-protein, UV absorbing peak eluted at two bed volumes

Figure 9: Desorption of Protein - Experiment 7

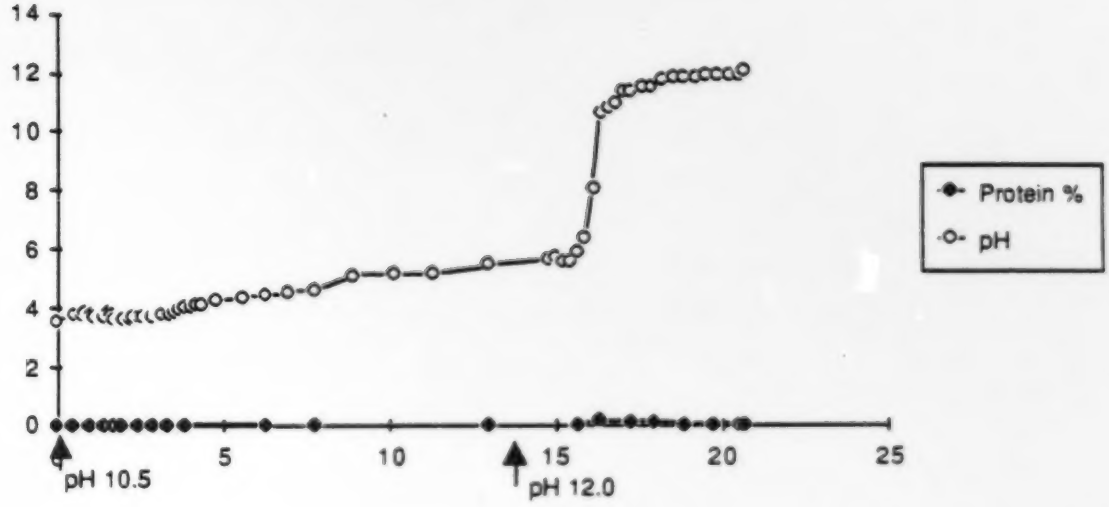
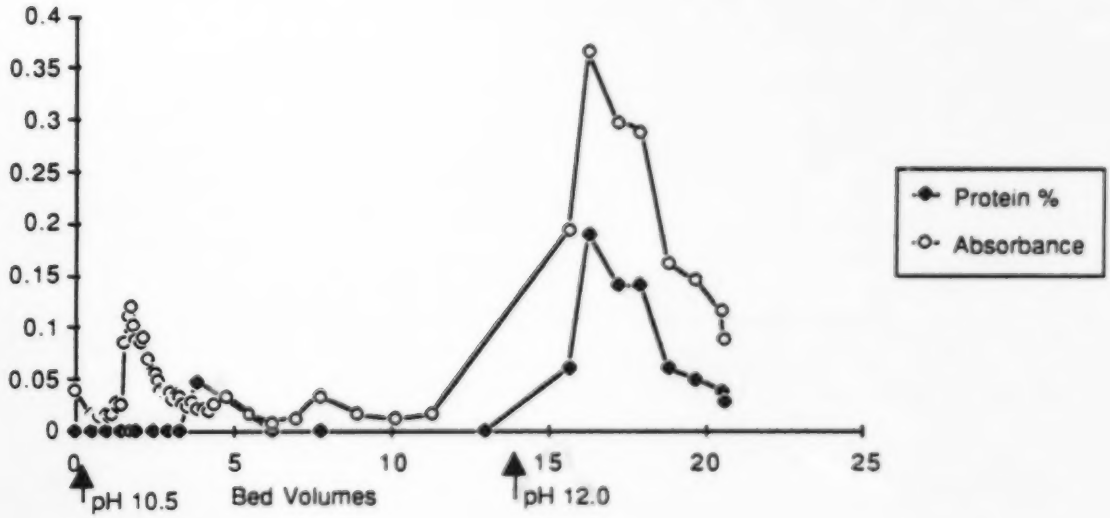


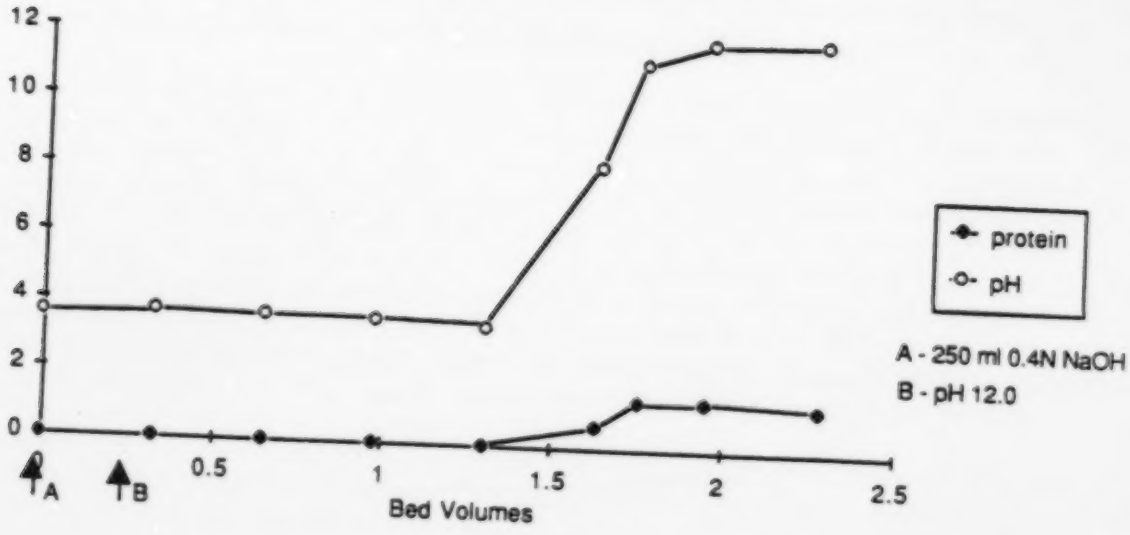
Figure 10: Desorption of Protein - Experiment 7



Experiment 8

250 ml of .4N NaOH was pumped onto a column prepared as above and then followed by pH 12.0 eluant. (figure 11) The bolus of caustic was not sufficient to exceed the buffer capacity of the bound protein however the same gelling effect occurred when the eluant elevated the pH. Again minimal protein was recovered.

Figure 11: Desorption of Protein - Experiment 8



General Discussion: Desorption

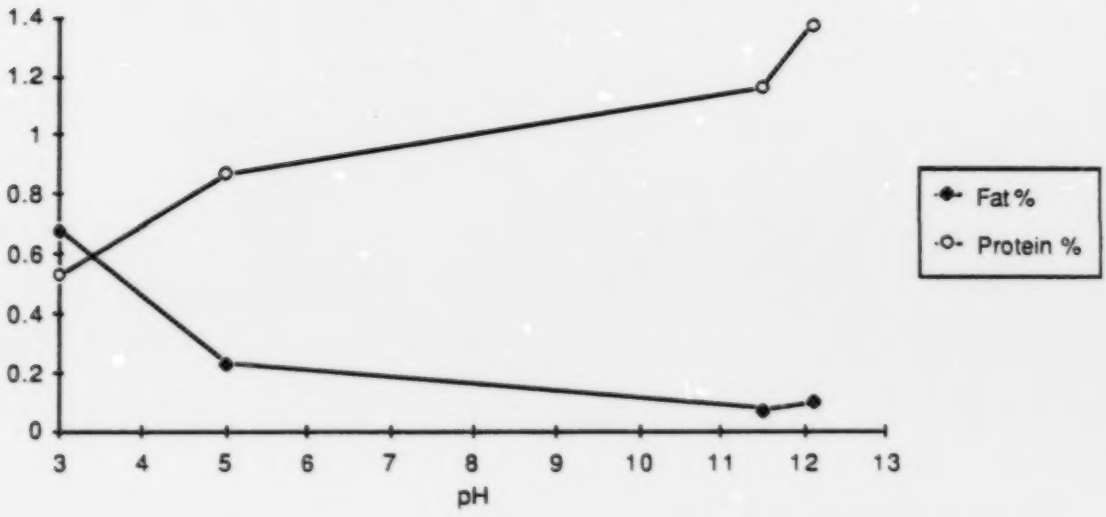
Under these experimental conditions the column process was not successful in eluting the protein in a commercially feasible manner. In order to achieve elution in a reasonable volume, sufficient caustic must be supplied to neutralize the buffer capacity of the protein on the entire column. Unfortunately, this means that material neutralized and eluted at the top of the column finds itself in an environment of high pH leading to its gelation. Two possible solutions would be to elute with very large volumes of pH 9.0 solution or to use a buffered eluant. The former is impractical due to the large volumes involved and the latter would effectively require another purification step to remove the buffer after elution. One variation might be to recycle eluant and continuously adjust its pH to 9.0 by caustic addition eventually eluting enough protein to act as a buffer. This was investigated and although elution was achieved without gelation, volume and time requirements negated its apparent usefulness. It was decided that given time constraints this problem would not be pursued further and a batch process would be used for desorption in the remainder of the experiments.

Discussion of Analytical Results

As analytical results from early experiments were examined some inconsistencies, particularly with the protein and fat results were noted. Further investigation showed that the Superscan methodology (the same as that use for measuring milk composition in Ontario) was very sensitive to the pH of the sample. This was of concern because the samples ranged in pH from 3 to 12. Figure 12 shows the values obtained for fat and protein concentration when the same whey sample is adjusted to various pH's. A 300% protein variance and a 700% fat variance is obtained across this range. It was decided to adjust all samples to pH 12 prior to analysis from experiment 3 onward. This approach

ensures that any observed changes are valid, however absolute quantitation of protein levels by this method is questionable.

Figure 12: Variance of Superscan Reading with pH of Sample



These difficulties also may explain the poor correlations achieved between UV absorbance data and the Superscan readings. Although inconsistencies were expected for the raw whey due to the presence of many non-protein UV absorbing substances, the correlation should have been much better for the relatively pure protein eluate. As a result, limited conclusions could be drawn from the UV data in these experiments.

In order to verify the validity of the protein recovery levels we observed, final products were back checked by subsequent Kjeldahl analysis in three different laboratories, using the A.O.A.C method for protein determination.

Additional verification of levels was obtained by performing true protein analysis (total nitrogen - non-protein nitrogen) on selected samples using the trichloracetic acid precipitation technique. Visual examination of the precipitates showed almost total removal of protein from the whey by the column. Superscan analysis showed less than 15% of the total nitrogen in effluent whey was from true protein while the corresponding number for demineralized whey was less than 7%. Protein eluate from regular whey was over 90% true protein while demineralized whey yielded an eluate with 100% true protein.

Process Simulation

Based on the encouraging column results from the initial experiments, a simulated moving bed system was set up to examine the media's performance. The system consisted of five 1 litre columns in series, operated in an up flow direction. It had been established in the previous experiments that resin shrinkage was proportional to protein load, and this was used as a initial measure of uptake. The experiment involved pumping 10 litres of

whey/hour through the columns at 32°C. Each hour the inlet column was removed and a fresh column placed on the outlet end of the series. Since continuous desorption was impractical, these columns were saved for batch elution at the conclusion of the experiment. The experiment was continued until the initial outlet column had worked its way through the entire cycle, thus being exposed to a total of 50 litres of whey.

On the first cycle the inlet column behaved identically to those under the same conditions in the single column tests, losing approximately 15% of its inital volume. The second column also exhibited some shrinkage, indicating it was picking up protein not adsorbed by the first column. This trend continued through the five cycles. At the conclusion, the first and second columns had reached the same level of shrinkage (20%), with the third and fourth showing decreasing amounts of volume loss, and the fifth exhibiting only slight shrinkage. This indicate that a column (or media batch in a commercial sense) would become fully saturated prior to removal for elution and that the following columns did act effectively as scrubbers for unadsorbed protein. This would be expected from the earlier results showing longer media contact (slower flow rates) results in increased recovery.

At the point of maximum protein loading, it was still possible to maintain the 10 litre/hour flow rate through the five columns with minimal resistance. Pressure drop across the five columns did not exceed 10 psi at any point in the experiment. The batch desorption of the resin indicated that it could be fully regenerated even from this high level of protein loading.

Based on these results it was concluded that the CMB process was sucessful for the adsorption of protein from the whey solution and would maximize the recovery of the material from solution.

Product Production:

As discussed earlier batch desorption became a necessity, and this combined with the need to produce large quantities of protein eluate led to the decision to use the batch process for both adsorption and desorption.

Conditions:	whey source	Mapledale cheddar
	whey initial pH	5.75

Whey on receipt was clarified and chilled to 4°C until use. Individual 60 litre batches of whey were pasteurized for 16 sec. at 72°C, cooled to 45°C, and adjusted to pH 3.0. The whey was then stirred with media for 30 min and drained. The deproteinated whey was collected and frozen for use in future lactose recrystallization studies. Media was rinsed twice with pure water and allowed to drain. Sufficient water was added to allow thorough stirring and the pH adjusted to 9.0. The mixture was allowed to desorb for one hour and then drained and rinsed with a minimum amount of water. The combined eluant was analyzed and refrigerated until further use.

Six batches of whey were processed by this methodology (the theoretical protein yield for these batches would be 2,160 gm) resulting in a apparent yield based on Superscan analysis of 81.6%.

One batch of demineralized whey adjusted to pH 3.0 was processed as above with an apparent protein yield of 70.3%. An additional 8 l rinse was performed after this experiment to determine the efectiveness of the standard rinsing steps. The results showed an additional 9.6% protein could be recovered, bringing total recovery to 77.8%.

In order to further investigate the properties of the gelled protein obtained in the early desorption studies, one batch was elevated to pH 11 where a large quantity of gel was formed. The batch was then titrated back to pH 9.0 with HCl to resolubilize the gel and the protein recovered in the normal fashion. Analysis indicated a theoretical yield of 32.0 % protein through this procedure.

Final Product Preparation

The protein eluants were dried to an approximate 5% moisture in a laboratory scale spray dryer utilizing an inlet temperature of 217°c and an outlet of 93°C. This process was prolonged because the material had such a low solids content (3%) however a suitable ultrafilter for preconcentrating was unavailable. Subsequent analysis of the weight, moisture and protein content of the control powder indicated an overall process recovery of 75.7% had been achieved.

Part 2 - Charactarization of Protein Product

Following the production of approximately 2.5 Kg of product at the Ontario Technology Centre, Protose conducted a number of studies at the University of Manitoba, Dept. of Food Science and the Agriculture Canada Food Research Institute to further charactarize the chemical and functional properties of the protein. Protein samples from regular whey (control), demineralized whey (demin) and resolubilized gel (altered) production batches were compared. In addition Protose enlisted the services of The Griffith Laboratories to perform similar studies on the control protein as an independent validation of results.

A protein content of 86.6% on a dry basis was achieved for the control batches as compared to a production goal of 97%. This shortfall is a result of several factors. The proposed commercial process would utilize an ultrafilter to preconcentrate the protein solution prior to spray drying. With ultrafiltration the salts and lactose pass freely throught the membrane and would be removed at a rate equal to water reduction. The calculated composition for the control powder had an ultrafilter been used to concentrate to 24% solids prior to drying is illustrated in the following table. It can be seen that the target product purity would be achieved if this process step is included. Additionally, in the batch procedure employed, rinse volumes in the adsorption step were approximately one-third of those used in the normal commercial process, resulting in higher level of impurities.

Compositional Analysis

	Control		Altered		Demin		UF Control*
	%	dry %	%	dry %	%	dry %	proj. dry%
Protein	81.7	86.6	82.2	89.5	76.5	82.7	97.7
ash	3.4	3.6	4.7	5.1	3.1	3.4	0.45
moisture	5.7	---	8.2	---	7.1	---	---
lactose	7.3	7.7	ND	---	ND	---	0.96
fat	1.1	1.2	ND	---	ND	---	0.9
Total	99.2	99.1	---	---	---	---	100.0

*calculated, ND = not determined

It may be noted that there is little apparent effect of demineralization on the ash content of the final product. This is because the majority of salts are washed away in the adsorption rinse stage, negating any initial differences. The final ash content is predominately determined by the amount of caustic employed to adjust the pH for desorption. The higher ash content in the altered batch results from the acid used to bring the pH back down after gelation.

SDS-PAGE Electrophoresis

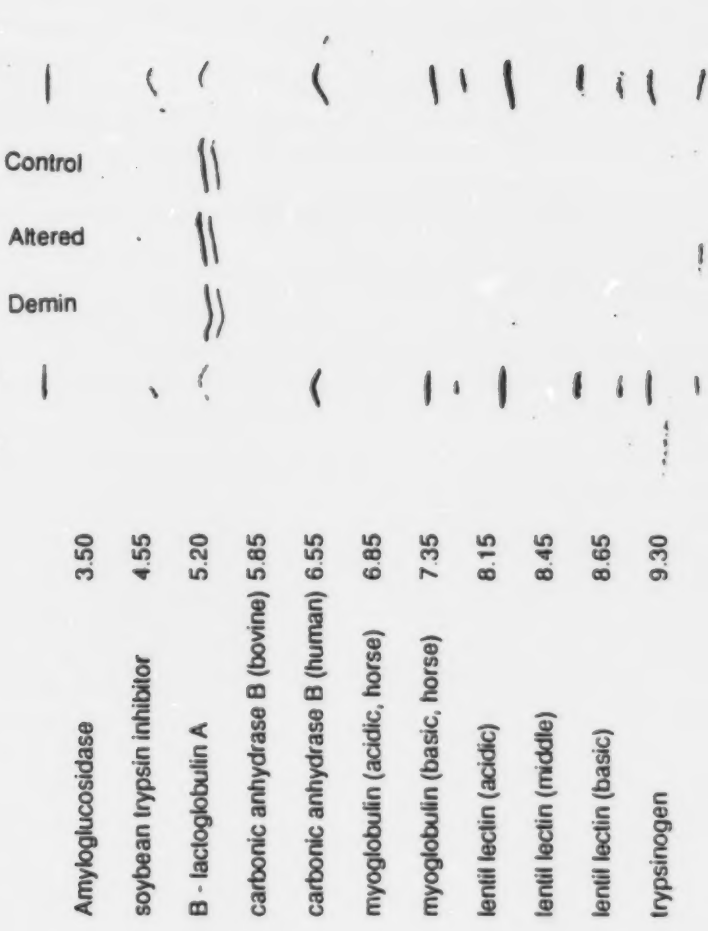
Analysis of the protein composition by electrophoresis indicated the majority of the protein was concentrated in two bands corresponding to molecular weights of 14,000 and 18,000.(see figure 13) These represent alpha-lactalbumin and beta-lactaglobulin respectively. A small amount of material at MW 66,000 corresponding to bovine serum albumin (BSA) was present in the control and altered samples but absent from the demin. This could be due to adsorption of BSA by the demineralization resin which could lead to potential fouling and regeneration problems. Alternatively the BSA may have been precipitated by the low pH conditions achieved in the cation exchanger.

Isoelectricfocusing

This technique yielded two very sharp bands corresponding to alpha-lactalbumin and beta-lactaglobulin as well as a third minor component at a pI<4.5. (see figure 14) This component was reduced in the demin sample relative to the other two and is likely to be BSA.

Figure 13:
SDS- Polyacrylamide Gel Electrophoresis

Figure 14:
Isoelectric focusing



Chromatography

Size exclusion chromatography was not able to resolve the two major components, however it also did not reveal the presence of any low or high molecular weight components in detectable quantities. Hydrophobic interaction chromatography (HIC) was able to separate alpha-lactalbumin (A) from beta-lactaglobulin (B) and revealed that the ratio of A concentration to B was increased significantly in the altered sample.(figure 15) A small peak of low hydrophobicity was noted in the control sample but not in the other two. Reverse phase chromatography was able to resolve the two components and comparison of peak areas revealed the same finding as with HIC.(figure 16) The area ratio of A to B was 0.889 in the control, 1.00 in the demin, and 1.33 in the altered. This could be a result of selective loss of the B fraction in the gelation process. Some loss may also be indicated by the somewhat higher ratio evident in the demin sample.

Spectroscopy

Comparison of the three samples by UV and fluoresence spectroscopy revealed no major differences in their spectra. (figure 17)

Color Determination

The three samples were compared to a standard white tile in a HunterLab color comparator. All samples showed a slightly higher yellow component than the standard but this was greatly reduced over that of a typical whey protein concentrate. The control and demin were equal to the standard in whiteness while the altered batch had a slight grey component.

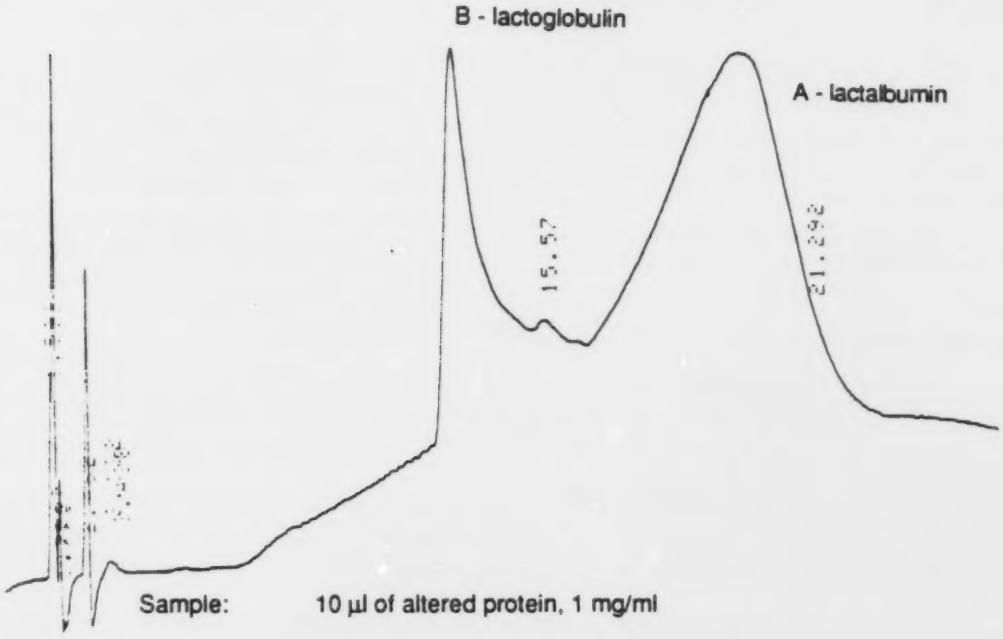
Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) profiles were obtained to determine the degree of denaturation that had taken place in the product.(see figure 18) The results for the three samples and published data for typical whey protein concentrate are as follows:

	Control	Demin	Altered	WPC
Temp. of Denaturation °C	63.50	63.24	67.07	85.26
Enthalpy of Denaturation J/g	8.89	6.04	5.28	1.92

The large enthalpy values indicate minimal denaturation has occurred during production of the protein and good functionality would be expected. The decreasing values with demin and altered indicate that some damage was done with these processes, although a suprising amount of functionality remains with the altered considering the rigorous treatment. The lower temperatures compared to WPC are a result of both higher purity and less denaturation in the protein isolate. The WPC used was 55% protein and the remaining lactose will contribute to a raised denaturation temperature.

Figure 15: Hydrophobic Interaction Chromatography



Conditions: Mobile phase A - 1.7 M $(NH_4)_2SO_4$ in 0.1M NaH_2PO_4, pH 7.0
Mobile phase B - 0.1M NaH_2PO_4, pH 7.0
Gradient from 0% B to 100% B over 20 min. at 1 ml/min

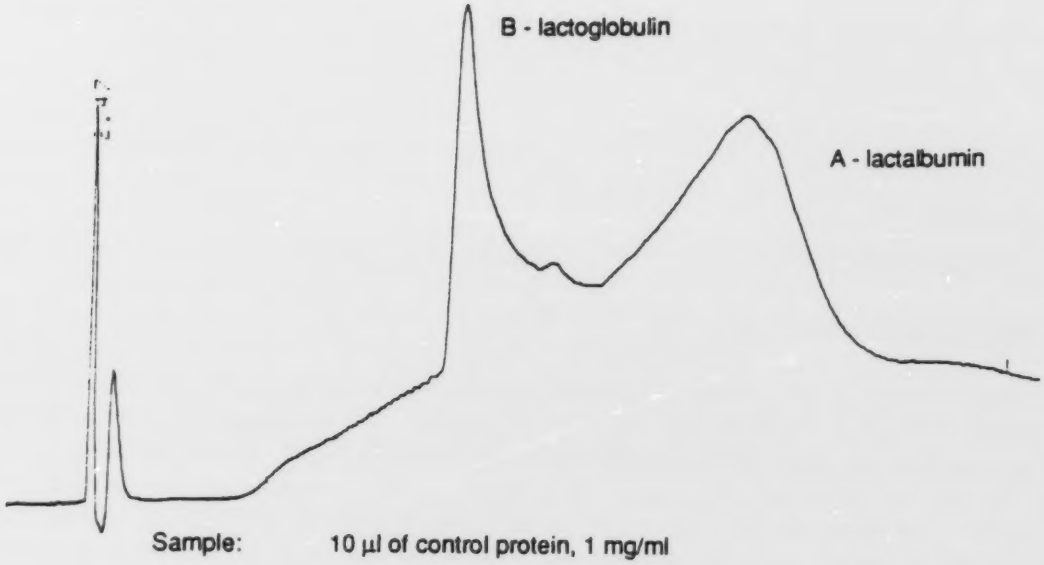


Figure 16: Reverse Phase Chromatography

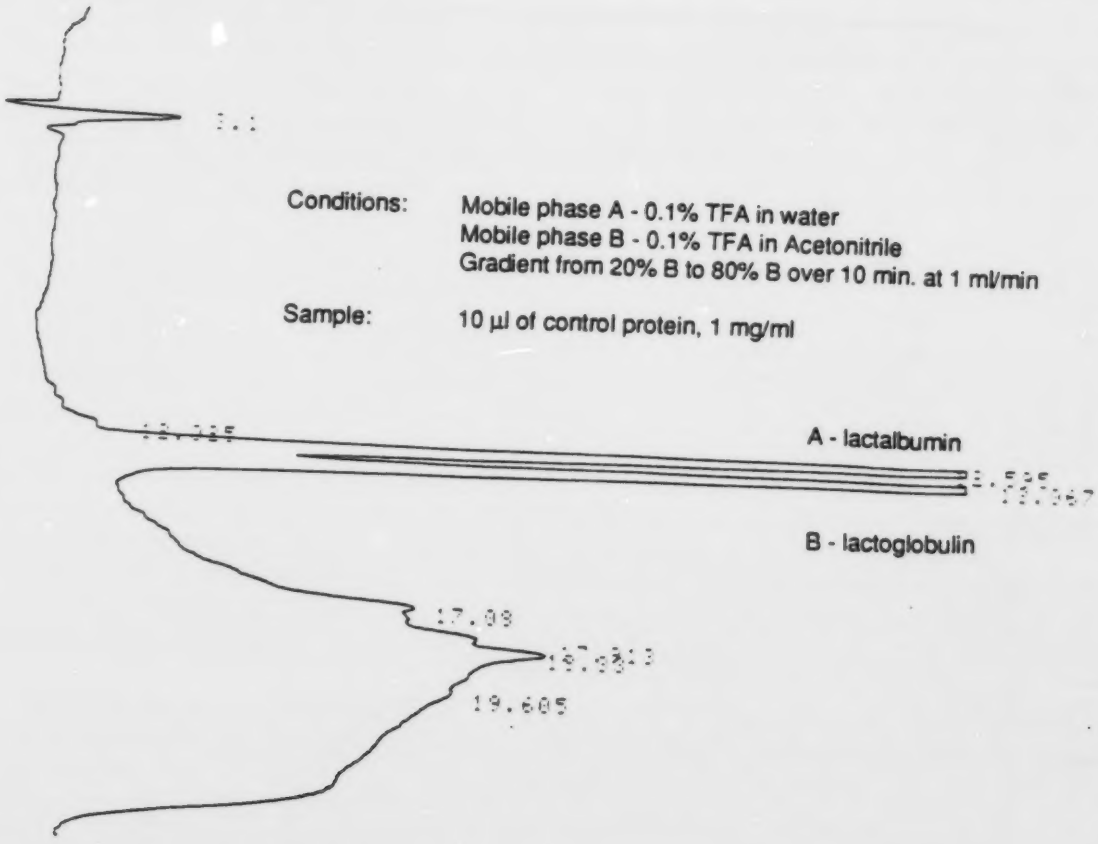
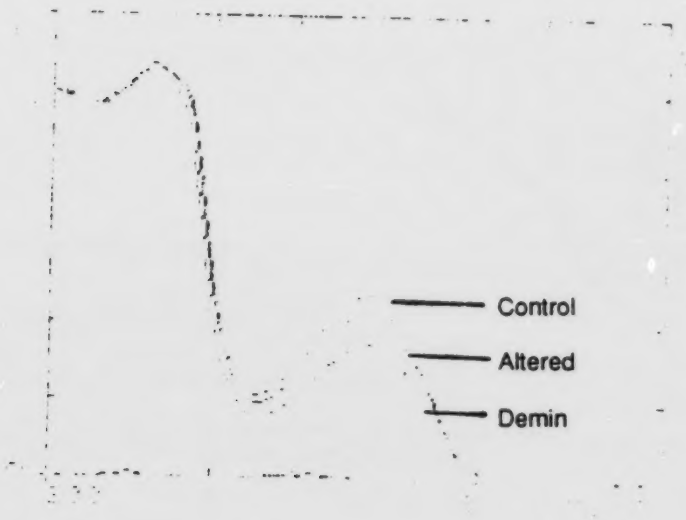

Conditions:
Mobile phase A - 0.1% TFA in water
Mobile phase B - 0.1% TFA in Acetonitrile
Gradient from 20% B to 80% B over 10 min. at 1 ml/min
Sample:
10 µl of control protein, 1 mg/ml
A - lactalbumin
B - lactoglobulin
17.08
19.605

Figure 17: Ultraviolet Scanning Spectroscopy



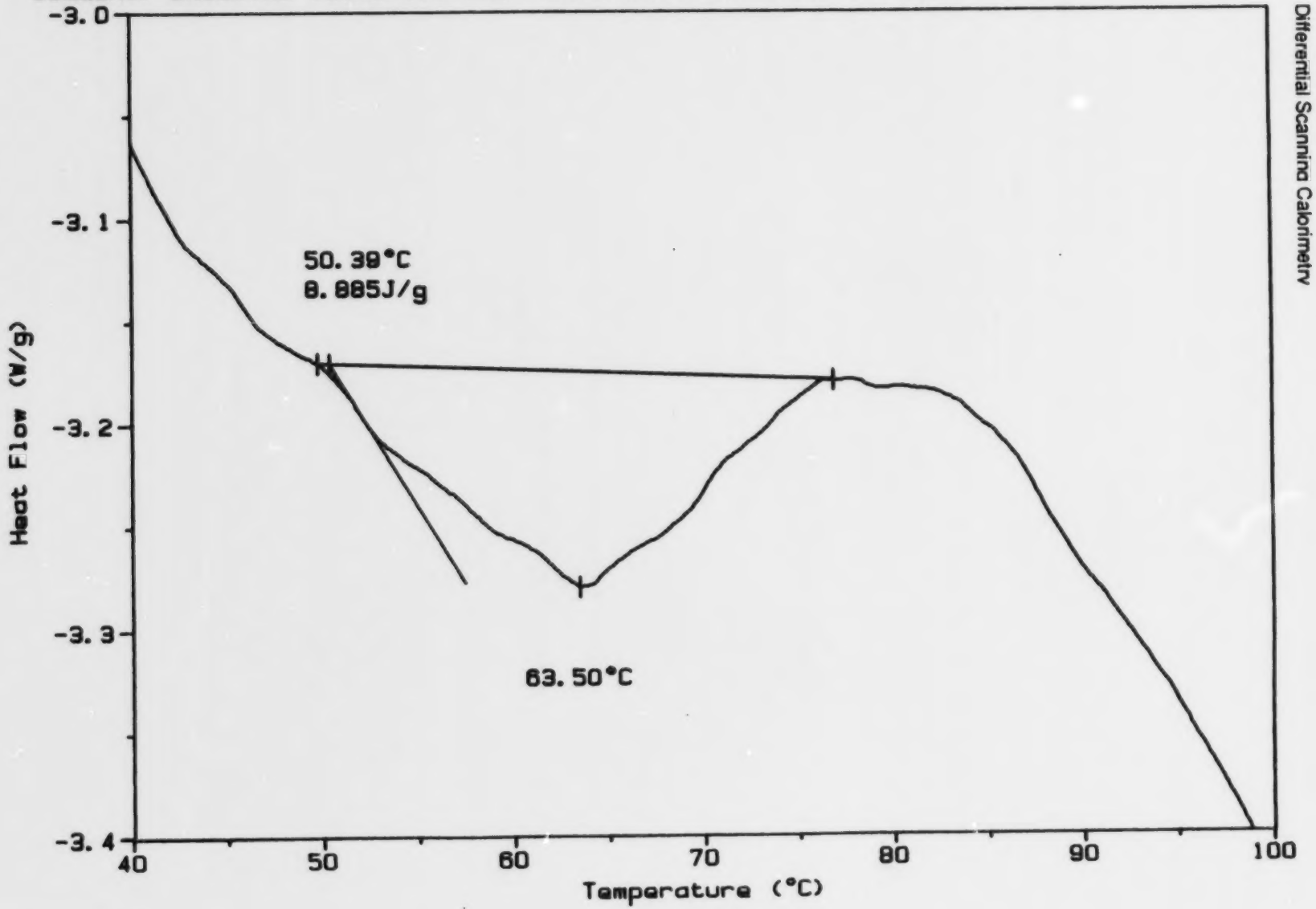


Figure 18: Differential Scanning Calorimetry

Rheology

In order to determine the gel formation properties of the product relative to food systems comparison tests against egg white were performed. Protein samples and egg albumin were dissolved in 0.1M NaCl at a conc. of 15% total solids and heat set in boiling water for 20 minutes. The resultant gels were sliced to a 2mm thickness and analyzed on a Bohlin VOR Rheometer. Elasticity and viscosity parameters were measured by the small amplitude oscillatory testing mode.

	G'	G"	tan delta
Control protein	32950	3275	.099
Demin protin	23100	2210	.095
Altered protein	41000	5380	.131
egg albumin	9765	1565	.160

G' is a measure of elasticity, G" is an indication of viscosity with all protein treatments showing significantly higher elasticity and viscosities than egg albumin. The tan delta or ratio of these components is an indication of overall gel structure, with lower numbers indicating better crosslinking and less granulation. The control and demin samples are clearly superior to egg white in this regard while the altered sample reflected the loss of some of its functionality.

Preliminary responses from outside functionality testing have also been very encouraging, further supporting the conclusion that the process is able to maintain the majority of protein functionality.

Part 3 - Lactose Crystallization

Studies to determine the ability to crystallize lactose from the deproteinized whey produced at Chatham were undertaken at The Food Research Centre in Ottawa. The procedures followed were identical to those published by Dr. H.W. Modler in 1986 (ref. 1) to allow direct comparison to data for regular whey. Results for deproteinized whey (DP), demineralized deproteinized whey (DM/DP) and regular whey were as follows:

Weight After Crystallization

	DP	DM/DP	Whey
Mother liquor (gm)	85.26	55.71	232.0
Crude lactose (gm)	310.52	340.25	158.0
Washed/dried lactose (1 wash, gm))	98.18	126.54	59.2
Washed/dried lactose (2 washes, gm)	94.92	108.12	---

	Mean % Protein		
	DP	DM/DP	Whey
Washed/dried lactose (1 wash)	1.22	1.64	1.0
Washed/dried lactose (2 washes)	1.54	1.01	---

	Mean % Ash		
	DP	DM/DP	Whey
Washed/dried lactose (1 wash)	1.15	0.49	0.4
Washed/dried lactose (2 washes)	1.26	0.35	---

Under identical conditions, the DP whey yielded 65% more lactose, and the DM/DP whey yielded 113% more dried lactose than untreated whey. The DM/DP lactose appeared to be more soluble as evidenced by greater losses with a second wash step. DP whey yields less pure lactose in terms of both protein and ash compared to regular whey. DM/ DP whey lactose is similar to regular whey lactose in ash content but higher in protein. It appears that the large increase in crystal yield results in the trapping of a higher % of impurities.

Part 4 - Projected Waste Discharges from a Commercial Plant

Untreated whey has a BOD level of approximately 30,000 mg/litre. The majority of this is associated with the lactose component of the waste. With this process 78% of the BOD load and 70% of the total solids will be recovered in the production of high value protein and lactose products. The remaining material will be contained in a volume equal to about 4% of the initially processed whey with a 33% total solids concentration. On a dry basis this will have a composition of 14% protein (3% true, 11% non-protein nitrogen), 33% ash and 53% lactose. The material can be utilized in liquid form as an animal feed energy enhancer or dried and sold for use as a binder in pelletized feed manufacturing. Some potential exists for pilot plant work to identify ways to further increase lactose recovery and reduce the quantites disposed of through this route.

The use of demineralization in the process would generate approximately 650 kg of sodium chloride in the regeneration waste stream for every 100,000 litres of whey processed. Optimization of the CMB process to eliminate the need for demineralization would result in signifcant waste disposal and operating cost savings.

Based on the high recovery of BOD content through the commercial processes discussed above, the primary source of waste will be the minor amounts of whey and detergents in the wash waters generated during cleaning and sanitization of the equipment. The use of a continuous process will help to reduce this volume as will the fact that most rinse waters will be reclaimable. Accurate quantitation of the volume and composition of this stream will require further work

Conclusions

As outlined in the Protose document dated September 29, 1987 the objective in conducting this experimental work was to determine the effectiveness of the ion exchange cellulose when utilized with Eastern Ontario cheese whey, as well as to verify that the preferential CMB process could be utilized. It was felt that if these points could be established with a reasonable degree of certainty then Protose could proceed with a commercial plant which would convert a major pollutant, cheese whey, into pure whey protein isolate and USP grade lactose.

As described in the preceeding pages, the work conducted was extensive and the conclusions were readily apparent in that the cellulose proved to be extremely effecient in extracting protein from the whey samples and that the adsorption portion of the CMB process was also very effective. What the testing also established was that significantly more work has to be done before the decision to proceed with a commercial scale plant can be made.

The outstanding issues that a pilot plant would have to resolve are as follows:

1) the development of an effective desorption process
2) further study on the demineralization process to conclusively determine if it is required
3) the monitoring of the complete CMB and lactose process to determine overall product recovery and pollution elimination rates when dealing with larger volumes
4) the generation of product to be used in a marketing program and to establish to potential customers that the product can be produced on a consistent basis

Protose plans to proceed with a pilot plant in order to address these unknowns in the second quarter of 1988.

Reference 1: "Influence of pH, Casein, and Whey Protein Denaturation on the Composition, Crystal Size, and Yield of Lactose from Condensed Whey., H.Wayne Modler and L.P.Lefkovitch, J. Dairy Sci. 69:684-697, 1986